Research Article

Infantile spasms in early-onset Niemann–Pick disease with a novel compound heterozygous mutations in SMPD1 gene

Massimiliano Chetta\textsuperscript{a,1}, Anna Guacci\textsuperscript{a,1}, Francesca Rizzo\textsuperscript{a}, Giovanna Marchese\textsuperscript{a,b}, Francesca Felicia Operto\textsuperscript{c}, Alessandro Weisz\textsuperscript{a,d,*}, Giangennaro Coppola\textsuperscript{c,**}

\textsuperscript{a} Laboratory of Molecular Medicine and Genomics, Department of Medicine and Surgery, University of Salerno, Baronissi, Italy
\textsuperscript{b} Genomix4Life Srl, Department of Medicine and Surgery, University of Salerno, Baronissi, Italy
\textsuperscript{c} Child and Adolescent Neuropsychiatry, Department of Medicine and Surgery and “SS. Giovanni di Dio e Ruggi d’Aragona - Schola Medica Salernitana” Hospital of the University of Salerno, Salerno, Italy
\textsuperscript{d} Molecular Pathology and Medical Genomics, “SS. Giovanni di Dio e Ruggi d’Aragona - Schola Medica Salernitana” Hospital of the University of Salerno, Salerno, Italy

\textbf{A R T I C L E  I N F O}

Available online 1 January 2016

\textbf{Keywords:}
Niemann–Pick disease
NPA
NPB
SMPD1
NGS
Exome sequencing

\textbf{A B S T R A C T}

Niemann–Pick diseases are a group of rare autosomal recessive disorders caused by an inherited deficiency of lysosomal storage with similar clinical presentations. At least three different Niemann–Pick (NP) diseases have been described, with NPA and NPB occurring as a result of a deficiency of the acid sphingomyelinase (ASM) enzyme, while NPC as a disorder that cause misregulation in cholesterol and lipids turnover, causing their accumulation in various tissues, including brain. The resulting phenotypic spectrum ranges from a severe infantile type with neurologic degeneration and death, usually by 3 years of age (NPA), to a non-neurologic adult onset form compatible with survival into adulthood (NPB) and a neurovisceral disorder with symptoms that occur at different times and progress independently (NPC).

Here, we report on an Italian child born from non-consanguineous healthy parents, with a negative family history, who developed infantile spasms at the age of 5 months and clinical signs of potential storage disease. The genetic screening, performed by means of whole exome sequencing, revealed compound heterozygous mutations in the Sphingomyelin Phosphodiesterase 1 gene (SMPD1), comprising both a homozygous polymorphism (p.V36A) in exon 1 and a new frameshift heterozygous deletion (c.1187delT) in exon 3 generating a premature stop (TAA) at codon 424 (p.L395fsX29).

This result appears to corroborate the phenotypic heterogeneity of the symptoms and suggests a correlation between the presence of a truncated SMPD1 polypeptide and the very early onset of the disease.

\textbf{Focal points}

\textbf{Benchside:} The comprehension of genotype–phenotype correlations in patients affected by Niemann–Pick disease will accelerate the accuracy of the diagnosis and permit to ameliorate patient follow-up.

\textbf{Bedside:} The coexistence of a homozygous polymorphism and of a new heterozygous frameshift deletion in exon 3 of the SMPD1 gene reveals the presence of infantile spasms, not previously related to mutations in SMPD1 gene. Elucidating the mechanisms associated to this altered gene product could open novel approaches in therapy.

© 2016 European Society for Translational Medicine. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Niemann–Pick (NP) disease refers to a group of inherited metabolic deficiencies caused by a rare and complex lysosomal storage disorder with similar clinical symptoms, divided into two major autosomal recessive sphingolipidoses: Niemann–Pick type A (NPA, MIM\#257200) and type B (NPB, MIM\#607616), caused by mutations in SMPD1 (sphingomyelin phosphodiesterase 1, MIM\#607608) gene, resulting in inherited deficiency of lysosomal acid sphingomyelinase (ASM, E.C. 3.1.4.12) [19] and

\textcolor{red}{http://dx.doi.org/10.1016/j.nhtm.2015.11.001}

2307-5023/© 2016 European Society for Translational Medicine. Published by Elsevier Ltd. All rights reserved.
Niemann–Pick types C1 and C2 (NPC1 MIM_#257220 and NPC2 MIM_#607625), due to the presence of mutations in NPC1 and NPC2 genes, respectively, that impair cellular processing and transport of low-density lipoproteins (LDLs) and cholesterol [17]. NP type D and E were also described as distinct entities, related to specific founder mutation of the NPC1 gene in a Nova Scotian group and to adult onset respectively, but at present these have been shown to belong to NPC.

SMPD1 gene maps on the short arm of chromosome 11 (11p15.4), it is composed of six exons, spans approximately 5 kilobases (kb) and encodes a glycoprotein of 629 amino acid [9]. The incidence of NPA and NPB combined, due to SMPD1 mutation, it is estimated to be one in 250,000 in the general population, with estimated birth rate of ~0.4–0.5 per 100,000 [15]. NPC 1 and 2 genes are located on the long arm of chromosomes 18 (18q11.2; NPC1) or 14 (14q24.3; NPC2) and comprise 25 and 5 exons, respectively. They encode two distinct transporters, that act in concert to allow cholesterol mobilization, and their impairment cause NPC, that has an estimated minimal incidence of 1/120,000 live births, with approximately 1 in 400 carriers in the general population.

NPA is an infantile neurodegenerative disease characterized by severe neurologic disturbances, massive hepatosplenomegaly, progressive psychomotor deterioration including hypotonia, rigidity and mental retardation. Symptoms usually develop by six months and death occurs in the first few years of life [2,23]. NPB, known as the visceral NP form, is sporadic and compatible with survival into adulthood. Commonly it is characterized by diffuse involvement of spleen, liver, and lungs and is free by neurologic manifestations [5]. Very early onset has also been reported, characterized by a clinical spectrum of neurological symptoms with mild developmental delay and visceral manifestations, determining an individual clinical evolution. Milder neurological symptoms with mild developmental delay and visceral manifestations, determining an individual clinical evolution [6].

According to the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php), 175 mutations have been identified in NPA and NPB patients, distributed along the SMPD1 gene (for details see below). Three of these mutations (p.L302P, p.R496L and c.330delC) account for ~95% of patients with NPA, and one recurrent mutation, c.608delCGG, is responsible for most cases of NPB in Ashkenazi jews [20]. Missense mutations are the most common abnormalities in SMPD1 gene, although nonsense, splicing, small insertion and deletion (InDels) mutations have also been reported. To date no pathogenic regulatory variants or large InDels have been described.

For NPC1 gene, 387 mutations have been reported, accounting for 95% of all NPC cases, while only 23 are known for NPC2 [1].

Here we report the results of whole exome sequencing analysis in a child with developed early-onset hepatosplenomegaly together with increasing generalized hypotonia and difficult sucking, who developed infantile spasms at the age of five months.

2. Materials and methods

2.1. Case report

The patient was a male, born at term by healthy non-consanguineous parents after normal pregnancy and caesarean section due to episodes of heart frequency slowdown in the last two days before birth. His mother was 29 years-old and his father 34 years-old. Family history was overall negative. Newborn body weight was 2,700 gr, with Apgar scores of 4 and 7 after 1 and 5 min, respectively. The newborn presented with diffuse cyanosis and mild wheezing in the first 12 h after birth. From the third day of life, the child showed increasing jaundice with values up to 18 mg of blood total bilirubin, which required several days of phototherapy; jaundice persisted until the end of the first month of age. Since the first months, an increasing generalized hypotonia with limb weakness was reported, together with slow sucking, difficult swallowing and risk of lung aspiration. The child began to present recurrent episodes of bronchitis, associated with failure to thrive. From the fourth month of life, a wide abdomen with gradual worsening hepatosplenomegaly was detected. At age 5 months, the child started with daily clusters of infantile spasms associated with an EEG hypersrrhythmic pattern, promptly controlled by 150 mg/kg/day of vigabatrin monotherapy. The bone marrow examination performed at 10 months showed the presence of Niemann–Pick type foam cells, while only a mild microcytic anemia was present at 13 months of age. Deficiency in activity of acid sphingomyelinase in cultured skin fibroblasts was found. From 6–8 months onwards, the child showed a delay of psychomotor development, with substantial inability to sit independently, together with decreased tendon reflexes mainly in the lower limbs. At 10 months, the ophthalmological examination revealed a cherry-red spot. Echocardiography and EKG did not show anything significant at this age; towards the end of the first year of life and thereafter the child showed a worsening constipation resistant to dietary and pharmacological treatments. At 18 months, due to an episode of bronchopneumonic infection with hyperthermia and pleural involvement with difficult breathing, the child came to exitus.

3. Exome sequencing

Genomic DNA was isolated from peripheral blood leukocytes at 14 months of life, and after excluding the presence of genomic structural variants by array-CGH, the DNA was used for sequencing library preparation.

The complete exome sequencing was performed exclusively on the proband while it was not completed in the parents for the lack of informed consent.

Exons were captured using the SureSelect Human All Exon v4 kit (50 Mb; Agilent Technologies) and libraries were sequenced on Illumina HiSeq2500 with 72-bp paired-end reads by the NGS services of Genomix4Life Srl (Baronissi, Italy). Sequence reads were aligned against hg19 reference genome using Burrows-Wheeler aligner (BWA) software and Sequence Alignment and Mapping (SAM) files were converted to Binary Alignment and Mapping files (BAM) using SAM tools [11]. Following duplicate reads removal by Picard MarkDuplicates module of Picard Tools (http://picard.sourceforge.net), total number of reads was 105,752,213 and mean exon call was 51.5%. Mapped reads around insertion/deletions (InDels) were realigned using the RealignerTargetCreator and IndelRealigner modules of Genome Analysis Toolkit (GATK), the BaseRecalibrator module were used for recalibration determining the covariates (such as read group, quality score, machine cycle and nucleotide context) affecting base quality scores in the BAM file [14]. From recalibrated bam files, variants were first called using the UnifiedGenotyper module of GATK and ANNOVAR (www.openbioinformatics.org/annovar/) was used to annotate variants for localization within the transcription unit and for other annotations (i.e. exonic, intronic, UTRs, for exonic: synonymous, non synonymous, stop gain/loss, nonframeshift or frameshift InDels, conservation between species, annotation and allele frequency in 1000 Genomes database and dbSNP137). For variant detection and analysis Enlis Genomics (trial version) (http://www.enlis.com/) software v1.7 was used with a Read Depth filter from 10 to 20 and a Quality Score of 30.
SNP variations reported in the dbSNP database (http://www.ncbi.nlm.nih.gov/) and/or the 1000 Genomes project (www.1000genomes.org) were excluded from further analysis. Novel variations and sequencing errors were also filtered out using in house exome WT controls subset data.

4. Validation by Sanger sequencing

Mutations were confirmed by Sanger sequencing of both DNA strands of the regions of interest, using the following primer pairs (same as for the PCR reaction): 5’-CGTGTAGGAAGCGCGACA-3’ (forward) and 5’-CAGATTGGCAGGTTGTT-3’ (reverse) for exon 1 and 5’-ATGCCTTACCTCACCACCA-3’ (forward) and 5’-GACCATGAGCTGAATCCCCA-3’ (reverse) for exon 3. PCR was performed by FastStart Taq DNA Polymerase kit (Roche Life Science, Indiana, USA) and sequencing was carried out with a CEQ 2000XL DNA analysis system apparatus (Beckman) by the Molecular Biology Service of Stazione Zoologica “Anton Dohrn” (Naples, Italy).

5. Results and discussion

NGS analysis showed a total of 1,114,872 possible variations found on the first level of analysis (Table 1); 1,094,733 polymorphisms were removed and, after a stringent filtering step and confirmation by Sanger sequencing, we defined the presence of a compound heterozygosity in the SMPD1 gene, with the subject carrying a homozygous polymorphism in exon 1 (c.107T>C) causing an amino acid substitution of valine to alanine (p.V36A), and a new heterozygous deletion in exon 3 (c.1187delT) introducing a premature truncation (TAA stop codon) after residue 424 (p.L395fsX29) (Fig. 1). For comparison, all SMPD1 gene mutations known to date are summarized in Additional Table A1.

The V36A homozygous polymorphism was previously described in two Chinese sibling patients with NPB, in association with a novel missense mutation (c.1564 A>G) that caused an amino acid substitution of asparagine to serine (N522S) in one of the five functional N-glycosylation sites of ASM [8]. Another amino acid substitution (N522D), causing a mutant ASM protein with 10% residual activity in vitro, was previously reported in the same position in a NPB patient with moderate hepatosplenomegaly but no neurological involvement [4]. The p.V36A variant can be classified as likely pathogenic (PP4) in accordance to Richards et al. [18].

The new heterozygous deletion found here in exon 3 of SMPD1 gene produces a truncated C-terminal by 205 amino acids and can be classified as null variant (PVS1) [18]. Functional analysis in vitro revealed that C-terminal truncation mutation induced a fully inactive protein [3,5].

The exome sequencing results led us to re-evaluate the clinical data, inducing us to converge towards a diagnosis of NPD type A. Our patient showed the combined presence of early signs including visceromegaly and thus, despite the fact that the p.V36A variant was described in association with other mutations in NPB cases, the clinical picture reminded more the NPA phenotype with respect with early onset, prominent hepatosplenomegaly, axial hypotonia later combined with bilateral pyramidal signs, moderate feeding problems and pulmonary involvement associated with an increasing impairment of respiratory function, with exitus around one and half years of age [16,21]. It is reasonable to assume that the frameshift mutation could have caused a severe NPD condition, resulting in production of non-catalytic enzyme, with the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Summary metrics of whole exome sequencing results.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Statistics</strong></td>
<td></td>
</tr>
<tr>
<td>Total reads</td>
<td>111,045,202</td>
</tr>
<tr>
<td>Uniquely mapped reads</td>
<td>87,976,145</td>
</tr>
<tr>
<td>Reads in targeted regions</td>
<td>69,736,969</td>
</tr>
<tr>
<td>Average coverage (fold)</td>
<td>78.02</td>
</tr>
<tr>
<td>Targeted regions</td>
<td>213,383</td>
</tr>
<tr>
<td>Average read length (bp)</td>
<td>68.53</td>
</tr>
<tr>
<td><strong>Summary of variations identified</strong></td>
<td></td>
</tr>
<tr>
<td>Total number of variations</td>
<td>1,114,872</td>
</tr>
<tr>
<td>Total rare variations</td>
<td>311,109</td>
</tr>
<tr>
<td>SNP</td>
<td>1,094,733</td>
</tr>
<tr>
<td>Missense</td>
<td>15,793</td>
</tr>
<tr>
<td>Nonsense</td>
<td>177</td>
</tr>
<tr>
<td>Frameshift</td>
<td>324</td>
</tr>
<tr>
<td>Inframe InDel</td>
<td>203</td>
</tr>
<tr>
<td>Insertion</td>
<td>9567</td>
</tr>
<tr>
<td>Deletion</td>
<td>10,572</td>
</tr>
</tbody>
</table>

Fig. 1. A schematic representation of the SMPD1 gene and variants identified in exon 1 (V36A: left) and exon 3 (p.L395fsX29: right) by comparing data relative to the patient (Pt) and control (Wt). The variants were all confirmed by Sanger sequencing.
residual catalytic activity from the normal allele being sufficient to mitigate the neurological complications of NPA. With respect to the co-occurrence of infantile spasms starting at the age of five months, to the best of our knowledge this is the first report of West syndrome in a patient with an early-onset of NPD type A. According to Vanier and Suzuki (1996) and McGovern and colleagues (2006), seizures may occur but are not a major sign in Niemann–Pick type A [12,22]. In this case, infantile spasms appeared in short clusters associated with a somewhat typical hypsarrhythmic pattern and both these abnormalities promptly responded to vigabatrin treatment.

In conclusion one or more mutant alleles occur frequently in SMPD1 gene and this condition is not a prerogative of specific ethnic or demographic groups, such as reported for the Ashkenazi Jewish population. Probably common selective agent could have increased the heterozygote frequency by differential survival and highest fitness in individuals who carry a defective lysosomal enzyme [10]. The identification of new heterozygote mutations in SMPD1 gene provides additional evidence sustaining the need to consider the existence of a range of residual acid sphingomyelinase activity in Niemann–Pick patients that, upon genotype–phenotype correlations, will permit a more accurate genetic counseling in newly diagnosed cases of NPD.

Conflicts of interest statement

None declared.

Ethical statement

The study was conducted according to high ethical standard. An informed consent was obtained for the parents of the child patient and the study was approved by the appropriate ethical committee.

Funding source statement

None declared.

Acknowledgments

Work supported by: Italian Ministry of Education, University and Research (Grant: BIBIOFAR PON03PE_00146_1), National Research Council of Italy (Flagship Project InterOmics), University of Salerno (Grant: FAR B 2012-14) and Fondazione ‘Umberto Veronesi’.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.nhtm.2015.11.001.

References